tion. Interfering with hnRNP I results in developmental defects in *Drosophila* and amphibian. We characterized the adult phenotype of brom bones, a zebrafish mutant deficient in hnRNP I, and found that hnRNP I plays a novel role in regulating intestinal homeostasis. Brom bones display a number of defects in the intestinal epithelium, including abnormal cell lineage development, uncontrolled intestinal cell growth, and a markedly increased Notch signaling activity. Our biochemical analysis demonstrates that hnRNP I inhibits Notch signaling by controlling the stability of the intracellular domain of Notch (NICD). In addition to its role in the adult intestine, we found that hnRNP I is expressed in the developing digestive system in the zebrafish embryo. Morpholino knockdown of hnRNP I in zebrafish embryos impairs the development of multiple digestive organs, including the liver, pancreas and intestine. Our results demonstrate that hnRNP I plays important roles in the digestive organ development and adult intestinal homeostasis.

doi:10.1016/j.ydbio.2010.05.262

Program/Abstract # 219
Tubular extension and cell epithelialization are coordinately regulated and influenced by adjacent tissues
Yuji Atsuta, Emi Ohata, Ryosuke Tadokoro, Daisuke Saitou, Yoshiko Takahashi
Grad. Sch. of Biosci., Nara Inst. of Sci. and Tech., Nara, Japan

Epithelial tubules are essential functional components in major organs of the body. When a tubular structure forms during development, cells undergo epithelialization and robust extension. We have asked how these morphological events are coordinated in three-dimensional environment. To address these questions, we have recently developed a novel model using Wolffian duct (WD, also called a nephric duct), the earliest basis for kidney formation. WD is a simple structure, and extends in an anterior-to-posterior direction as a straight cord. Time-lapse imaging analyses revealed that cells located at the extending-front (tip region) are actively motile with numerous filopodia whereas cells residing in the rear region are epithelial in shape with less motility. Remarkably, when replaced into the front region, the rear cells can be converted to the front cell-like and restarted to extend posteriorly. These observations suggest that tissues surrounding the front region play instructive roles in the tubular extension. To further elucidate the molecular mechanisms underlying the tubular extension, we investigated the role of the chemokine SDF-1. SDF-1 (ligand) is expressed in tissues adjacent to the front cells of WD, which express the receptor CXCR4. When ectopically administered, SDF-1 attracted WD cells, suggesting that the WD extension is controlled by interactions between the neighboring tissues where SDF-1/CXCR4 signals instruct the front cells.

doi:10.1016/j.ydbio.2010.05.263

Program/Abstract # 220
Wnt4 induces tubule formation in metanephric mesenchyme by a non-canonical mechanism
Shunsuke Tanigawa, Honghe Wang, Yili Yang, Nirmala Sharma, Terry Yamaguchi, Alan Perantoni
Cancer Dev. Biol. Lab., NCI, Frederick, MD, USA

Wnt4 and βcatenin are both required for nephrogenesis, but studies of TCF-reporter mice suggest that canonical activation does not occur in metanephric mesenchyme (MM) during its conversion to nephronic epithelia. To study the mechanism, we developed a model that permits progenitor propagation in primary explant culture. Using this, we found that recombinant Wnt4 protein induces tubule formation and differentiation markers *Lim1* and *E-cadherin* in MM cells but does not activate a TCF reporter or expression of canonical Wnt target gene axin2 and minimally affects stabilization of βcatenin, which remains phosphorylated. Furthermore, Wnt4 caused localization of ZO1 and occludin to tight junctions. On the other hand, canonical activation with a TCF-βcatenin fusion construct, stabilization of βcatenin with a proteasomal inhibitor, or treatment of cells with a Wnt agonist, all of which activated a TCF reporter, were unable to induce tubule formation, and canonical Wnt inhibitor dkk1 could not block differentiation. Since a canonical mechanism is not operative in tubule formation, we assessed the role of non-canonical mechanisms with small molecule inhibitors. Both CaMKII and JNK inhibitors blocked tubule formation, and treatment of MM cells with Wnt4 caused a rapid activation of CaMKII and JNK. These results demonstrate that the canonical Wnt pathway is not responsible for mesenchymal–epithelial transition in nephron formation and suggest that both the non-canonical calcium–Wnt and the JNK-mediated Wnt/PCP pathways are involved in Wnt4-induced tubulogenesis in the kidney.

doi:10.1016/j.ydbio.2010.05.264

Program/Abstract # 221
Tissue interactions during formation of the pronephric duct in *Xenopus laevis*
Ian Hakkinen, Amanda Pinto, Julie Drawbridge
Dept. of Biology, Rider University, Lawrenceville, NJ, USA

In *Xenopus laevis* embryos, formation of the excretory system occurs in three temporally distinct phases: From stages 22 to 26 the pronephros and pronephric duct (PD) rudiments can be observed segregating from intermediate mesoderm directly ventral to somites IV–IX; from stage 29–33 cell migration extends the PD posteriorly from the level of somite IX to somite XIV; from stages 30 to 38 rectal diverticulae (RD) extend anteriorly from the cloaca to meet and fuse with the PD ventral to somite XIV. We have performed a set of tissue extirpation and tissue marking experiments to 1) investigate the tissue interactions required for maintenance and elongation of PD and RD tissue, and 2) determine whether cells from tissues other than the PD primordium contribute to the elongating PD. Tissue extirpation studies show that the epidermis is essential for maintenance of the PD and that removal of the PD does not prevent anterior extension of the RD, but is required for subsequent RD maintenance. Tissue marking studies indicate that neural crest does not contribute cells to the PD during PD elongation; removal of trunk neural crest results in foreshortening of the embryo but does not interfere with PD elongation. In addition, the width of the PD does not decrease as the PD elongates, indicating that the embryo can regulate PD size and may recruit cells from surrounding tissues.

doi:10.1016/j.ydbio.2010.05.265

Program/Abstract # 222
A role for GDNF in pronephric duct cell migration in *Xenopus laevis*
Erin McCafferty, Vanessa Gerrard, Nicole Revere, Julie Drawbridge
Dept. of Biology, Rider University, Lawrenceville, NJ, USA

Anterior to posterior extension of the *Xenopus* pronephric duct (PD) is complex, consisting of three distinct temporal phases: During the first phase, pronephric and PD tissue segregates from flank mesoderm directly ventral to somites IV–VIII; during the second phase, cells migrate throughout the duct extending it to the axial level of somite XIV; finally, anterior extension of rectal diverticulae (RD) from the cloaca to the posterior tip of the PD is required to

complete morphogenesis of a functional conduit for excretory waste. Our studies of axolotl embryos showed that GDNF signaling through the Ret/GFRalpha-1 receptor plays a role in posterior PD extension; we are extending our studies to investigate whether GDNF plays a similar role in *Xenopus*. Here, we show that *Xenopus laevis* expresses two GDNF paralogs similar to the long form of mammalian GDNF, and one alternatively-spliced form. We also show that GFRalpha-1 is necessary for the second phase of PD elongation. In addition, the expression patterns of *Xenopus* GDNF, GFRalpha-1 and Ret indicate that this signaling system could play a role in both PD and RD morphogeneses. Our strategy for testing whether GDNF is a PD or RD chemoattractant will also be discussed.

doi:10.1016/j.ydbio.2010.05.266

Program/Abstract # 223
Isoform and domain dependence of nonmuscle myosin II *in vivo* and *in vitro*
Aibing Wang[a], Xuefei Ma[a], Mary Anne Conti[a], Chengyu Liu[b], Sachiyo Kawamoto[a], Robert S. Adelstein[a]
[a]*Lab of Molecular Cardiology/NHLBI/NIH, Bethesda, MD 20892, USA*
[b]*Transgenic Mouse Core Facility/NHLBI/NIH, Bethesda, MD 20892, USA*

Nonmuscle myosins (NMs) II-A and II-B are essential for embryonic mouse development, but their specific roles are not completely defined. Here we examine the isoforms and their domain specificities *in vivo* and *in vitro* by studying mice and cells in which nonmuscle myosin heavy chain (NMHC) II-A is genetically replaced by NMHC II-B or chimeric NMHC IIs that exchange the rod and head domains of NM II-A and II-B. In contrast to the failure of visceral endoderm formation resulting in embryonic day (E)6.5 lethality of A^{-}/A^{-} mice, replacement with NM II-B or chimeric NM IIs restores a normal visceral endoderm. This is consistent with NM II's role in cell adhesion and also confirms an essential, isoform-independent requirement for NM II in visceral endoderm function. The knock-in II-B and chimeric mice die between E9.5 and 12.5 due to defects in placenta formation associated with abnormal angiogenesis and cell migration, revealing a unique function for NM II-A in placenta development. *In vitro* results further support a requirement for NM II-A in directed cell migration and focal adhesion formation. These findings demonstrate an isoform-specific role for NM II-A during these processes, making replacement by another isoform, or chimeric NM II isoforms less successful. The failure of these substitutions is not only related to the different kinetic properties of NM II-A and II-B, but also to their subcellular localization determined by the C-terminal rod domain. These results highlight the functions of the N-terminal motor and C-terminal rod domains of NM II.

doi:10.1016/j.ydbio.2010.05.267

Program/Abstract # 224
Characterization of an Ankyrin Repeat Socs Box gene in the early heart development of the basal chordate, Ciona intestinalis
Arielle Woznica, Brad Davidson
Dept. of Molecular and Cellular Biology, Molecular Cardiovascular Research Program, University of Arizona, Tucson, AZ, USA

FGF signaling drives the specification of heart precursor cells through the activation of an Ets family transcription factor (Ets1/2) in the basal chordate, Ciona intestinalis. Microarray analysis of gene expression from sorted heart lineage cells has led to the identification of candidate FGF/Ets target genes. I am currently studying the function and regulation of the FGF/Ets target gene Ankyrin Repeat Socs Box (ASB). In situ data shows ASB mRNA uniquely expressed in the heart precursor cells shortly after the start of neurulation. Functional analysis of ASB through misexpression suggests that ASB plays a significant role in heart development. Under conditions in which the signaling pathways controlling heart specification are blocked, the expression of ASB in heart precursor cells is sufficient for the partial rescue of heart cell migration. Analysis of a minimal enhancer region of ASB indicates that ASB is a direct target of transcription factors Ets1/2 and FoxF. Functional analysis of ASB will help determine the specific role of ASB in Ciona heart development. Detailed analysis of the regulatory elements driving the expression of ASB will help define the precise impact of FGF/Ets on the heart gene regulatory network, allowing us to identify and isolate the regulatory regions of other predicted FGF/Ets target genes utilizing a bioinformatics approach.

doi:10.1016/j.ydbio.2010.05.268

Program/Abstract # 225
Critical functions of myocardial Mycn in the developing mouse heart
Cristina M. Harmelink, Kai Jiao
Dept. of Genetics, Univ. of Alabama at Birmingham, Birmingham, AL, USA

The focus of this study is to define the role of the transcription factor *Mycn* in the developing mouse myocardium. Haploinsufficiency for *MYCN* causes Feingold syndrome, a developmental disease characterized in part by congenital heart defects (CHD). Mice lacking *Mycn* also display complex and variable CHD. Furthermore, *Mycn* is a target of Bone Morphogenetic Protein (BMP) signaling in the developing mouse heart. Disruption of BMP pathways results in abnormal development of the heart wall, valves and septa. Thus, Mycn is implicated in key cardiogenic processes but its exact functions remain to be established. I am testing the **hypothesis that Mycn is an essential regulator of mouse cardiomyogenesis**. Using conditional gene inactivation, *Mycn* is removed from the myocardium of mutant embryos. *Mycn* deletion was confirmed with semiquantitative PCR, western blot, and immunohistochemistry assays. In addition, western blot analyses reveal decreased expression of *Mycn* targets, *Ccnd1* and *Id2*. Loss of myocardial *Mycn* results in underdeveloped ventricles and embryonic lethality by E12.5, likely due to cardiovascular insufficiency caused by decreased ventricle contractility. Normally, ventricle chambers grow via cardiomyocyte proliferation and differentiation into muscular projections called trabeculae. The defective ventricle morphogenesis in mutants suggests a crucial role for *Mycn* in cardiomyocyte proliferation, survival and/or differentiation. Indeed, loss of *Mycn* causes a significant decrease in proliferation at E9.5. To discover the other effects of *Mycn* depletion, I am examining apoptosis and the expression of genes involved in ventricle morphogenesis.

doi:10.1016/j.ydbio.2010.05.269

Program/Abstract # 226
Mice null for Crim1 display altered BMP/TGFβ signaling, defects in multiple organ systems and die in utero with severe cardiovascular defects
David J. Pennisi[a], Han S. Chiu[a], K.S. Vinay[a], Lorine Wilkinson[a], Pumin Zhang[b], Melissa H. Little[a]
[a]*Institute for Molecular Bioscience, The University of Queensland, Australia*
[b]*Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA*

The purpose of this study was to determine what role the developmentally expressed molecule Crim1 plays in organogenesis. Crim1 is a trans-membrane protein capable of binding a range of